REMARKS/ARGUMENTS

Compliance with the sequence listing requirements of 37 CFR 1.821(a)(1) and (a)(2).

Applicants are submitting a substitute sequence listing, and Applicants request entry of the substitute sequence listing into the specification in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-23, in computer readable form (CRF), and a paper copy of the sequence information which has been printed from the floppy disk. The information contained in the computer readable disk was in part prepared through the use of the software program "PatentIn 3.0" and is identical to that of the paper copy.

This amendment contains no new matter. The additional sequences 5 and 6 find support in Figure 5 and paragraph [0013] of the original specification. Sequence 13 finds support in Tables B and C (paragraphs [0121] and [0122]) of the original specification.

Applicants have amended the specification to identify the sequences at page 45 and elsewhere correct deficiencies in the sequence listing. Accordingly, the Applicants and respectfully request reconsideration and withdrawal of this grounds of objection.

Status of the claims

Claims 143 to 146, 148 to 153, 188 to 190 were previously presented for examination on the merits. Claims 143, 144, and 188 to 190 are amended. Claims 191 to 193 are newly presented. After entry of these amendments, claims 143 to 146, 148 to 153, and 188 to 193 will be presented for examination on the merits.

Support for the amendments to the claims

Claims 143 and 144 were amended to add the limitation of previous claim 188 and to provide additional clarity by reciting "at least" as discussed further immediately below. Support for the 85% recital is found in the specification at page 22, second paragraph.

Claim 188 was amended to depend from claim 144 and to set forth a 90% sequence identity. Support for the 90% recital is found in the specification at page 22, second paragraph.

Claims 189 and 190 were amended to correctly set forth their base claim.

New claims 190 to 193 find support in original claims 143 and 145 and in the specification at page 22, second paragraph.

Accordingly, the Applicants believe the amendments to the claims add no new matter and respectfully request their entry.

Terminal disclaimers

Once the application is otherwise deemed to be in condition for an allowance, the Applicants intend to provide the required terminal disclaimers in order to overcome the various double patenting rejections.

Response to the objection of claims 188-190 under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim.

Applicants have amended claims 188, 189 and 190 to depend from claims 144, 143, and143, respectively.

The Action alleged that claims 188-190 expanded the scope of the claim from which they would depend. Applicants believe the allegation is based upon an improper interpretation of the base claims. Despite the "substantially identical" recital of claim 143 and the definition of the "substantially identical" term at page 22, second full paragraph, the Action would construe the previous version of Claim 143 as being limited to SEQ ID NO: 2 subject matter differing *only* by the possible exceptions cited in (i)and (ii). However, the term only is not found in the claim. In this regard, the Applicants' note that previous claim 143 did *not* recite "only differs from SEQ ID NO:2 by" or differs from SEQ ID NO:2 by *only*." Accordingly, it would have been more appropriate to construe the base claim more broadly, especially, given the recitals of the dependent claims which would require such.

Nevertheless, without acquiescing on the merits and in order to expedite prosecution of the application, the Applicants have amended base claims 143 and 144 to recite "differs *at least* from SEQ ID NO:2 by." Applicants believe this amendment resolves any concern on this point, and respectfully request reconsideration and withdrawal of this grounds for objection.

Response to the rejection of Claims 188 and 189 under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement.

As noted by the Examiner, whether undue experimentation is required to practice an invention is typically determined by the *Forman* factors. These factors weigh (i) the relative skill of those in the art; (ii) the nature of the invention; (iii) the breadth of the claims; (iv) the amount of guidance presented; (v) the presence of working examples; (vi) the state of the art; (vii) the predictability of the art; and (viii) the quantity of experimentation necessary. *Ex parte Forman*, 230 U.S.P.Q. 546 (PTO Bd. Pat. App. & Inter. 1986), *In re Wands*, 858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988).

The Action is based on the proposition:

> knowledge regarding the effects of multiple mutations up to 36 or 24 amino acid residues on the ability of the protein to fold and form the chromophore is lacking. Thus, searching for variants of SEQ ID NO: 2 having up to 24 or 36 amino acid variants which include insertion, deletion, substitution or combination thereof, or identifying a natural variant having 85% or 90% variants of SEQ ID NO: 2 is well outside the realm of routine experimentation and predictability in the art of success is extremely low. The amount of experimentation to identify a protein variant of SEQ ID NO: 2 having 85% or 90% amino acid homology from a natural or man-made is enormous. Since routine experimentation in the art does not include screening large numbers of gene and DNA library constructed from a natural source or man-made where the expectation of obtaining the desired gene is unpredictable, the Examiner finds that one skilled in the art would require additional guidance, such as information regarding the effects of varying up to 10 or 15% of the amino acid residues on the integrity of the protein and its ability to fold and form the chromophore. Without such guidance, the experimentation left to those skilled in the art is undue."

The Applicant addresses each of the Forman factors, particularly as raised above by the Action, and thereafter provides a general summary.

(i) Level of Skill in the Art.

Applicants believe that the relative skill and experience of those in the art of protein engineering is very high. Such work is typically conducted by research enterprises populated with persons with advanced degrees and extensive training in the relevant fields. Support for this assertion is readily evidenced by the authorship of the art disclosed with the previous IDS and discussed herein.

(ii) Nature of the Invention.

The invention is in the field of protein engineering. More particularly the invention is in the field of modified green fluorescent proteins. It is a field in which the measure of activity - fluorescence is extremely easy to monitor and the art of record has repeatedly evidenced the relative ease with which the protein moieties can be reengineered (see, Cubitt *et al., Trends in Biochemical Sciences 20(11):448 455 (1995), already of record).*

(iii) Breadth of the Claims.

Without acquiescing on the merits, and in order to expedite prosecution of the Application, the rejected claims have been amended in order to expedite prosecution of the application. Independent claims 143 and 144, as amended, now set forth a sequence identity of at least 85%. Dependent claims 188 and 189 set forth sequence identities of at least 90%.

Importantly, it should be further noted that the recital of independent claims 143 and 144 require at least six specific mutations as set forth in the claims. Accordingly, some portion of the percent differences is attributable to such specified mutations.

(iv) Amount of Guidance Presented.

As noted in the Action, the specification provides adequate guidance for all manipulations required to practice the invention.

The Action was particularly concerned as to the amount of guidance in the specification with respect to the effects of amino acid substitutions:

> With respect to the amount of guidance the Action alleges that one skilled in the art would require additional guidance, such as information regarding the effects of varying up to 10 or 15% of the amino acid residues on the integrity of the protein and its ability to fold and form the chromophore. Without such guidance, the experimentation left to those skilled in the art is undue."

In this regard, it is important to note that the Applicants have thoroughly characterized the primary, secondary, and tertiary structures of the referenced protein and how its functional domains operate. The specification teaches the 3D molecular structure of the protein down to the finest detail (see, Figures 1A, 1B, 2A, 2B, 2C, 5-1 to 5-45, 9 to 14) and exemplifies just how such information can be used in analyzing the crystalline structure of GFP and actually indicates where mutations in the molecule can alter the fluorescent properties of the molecule (starting at page 29 of the specification). The Applicants have shared the atomic model used to accomplish this (see, page 12, starting at line 17) by placing it on deposit in the Protein Data Bank (see, page 65, lines 22 to 23). The specification further expressly teaches the functional regions of the molecule in tables B, C, D, E, and F, including the role of various positions and various substitutions which can alter fluorescent properties. The specification discloses further a number of conservative substitutions which are less likely to affect fluorescence (see, page 21).

(v) Working Examples.

The specification further describes more than 20 amino acid positions which have a variety of suitable mutations (see, first full paragraph on page 8 to page 9, line 19). The specification discloses a number of individual constructs having a combination of five, six or seven of these mutations therein (see, table A at page 21, see, particularly, clone W2) and provides a working atomic model as to how to find or add other suitable mutations. Accordingly, the specification has working examples with combinations totaling about 3% actual substitutions and describes a source list of possible mutations which covers about 10% of the pertinent amino acid sequence. The above principally relates to combinations of advantageous mutations and does not address combinations with easier-to-find substitutions which may be compatible with function, if not necessarily advantageous.

(vi) State of the Art.

The state of the art is high. The methods for generating fluorescent protein moieties for use according to the invention are exemplified by the Heim et al. reference and the Applicants' own specification. This state of the art, when coupled to the Applicant's disclosure, provides one of ordinary skill in the art with the information and tools needed to broadly practice the invention as claimed.

(vii) Unpredictability of the Art.

As noted by the Action, the field of protein structure and function is generally one in which unpredictability can be a concern. However, green fluorescent proteins are amongst the most studied, characterized and reengineered of proteins (see, IDS setting forth a number of patents in this field). The Applicants have cited the Heim et al. reference which taught the critical portions of the fluorescent green protein and have incorporated by reference others (e.g., PCT/US95/14692) which expanded upon those teachings and evidenced many useful modified green fluorescent proteins of SEQ ID NO:2 that are already available.

Whatever is alleged to be the state of general unpredictability in the art, *multiple* suitable mutations were readily found for *each* of a number of individual amino acid positions (see page 9, lines 3 to 19) which are important to fluorescence. This finding, coupled with the other disclosures in the specification, make it quite reasonable to expect that multiple suitable substitutions, especially conservative ones, could be *at least* as readily obtained at the less important amino acid positions in the protein.

(viii) Undue Experimentation.

The Action has alleged that this is a field which "does not include screening large numbers of gene and DNA library constructed from a natural source or man-made." Applicants respectfully disagree. Using random mutagenesis techniques, Heim et al. screened six *thousand clones* in identifying a number of variants of interest (see, Heim et al. page 12503, last three lines of left column. Delagrave et al. (also of record) screened *30 thousand* clones in identifying their mutants (see, page 152, top of right column).

Given the ability to find multiple suitable substitutions at a great many positions, as evidenced in the specification, it would be comparatively simple for one of ordinary skill in

the art to start with such a protein sequence having a plurality of the advantageous modifications such as described in the specification and further modify the sequence by obtaining variants having additional substitutions in other portions thereof.

Additionally, using random mutagenesis methods, one of ordinary skill need not identify all such mutations in one step as they can obtain mutations in the required scope merely by starting with one of the Applicant's disclosed combinations and then serially subjecting them to steps of random mutagenesis and screening.

Accordingly, the quantity of experimentation necessary[1] to practice the invention with exemplified and non-exemplified embodiments is what is routinely performed by a person of ordinary skill in the art as illustrated in the instant specification and, for instance, recognized in U.S. Patent No. 6,803,188, U.S. Patent No. 5,777,079, and U.S. Patent No. 5,981,200. The relative ease by which suitable mutants for the fluorescent protein moieties of the constructs can be obtained and detected greatly reduces the experimental effort required to obtain constructs according to the invention.

(ix) Summary and Overall *Forman/Wands* Analysis.

As set forth in the MPEP §2164.01(a), the final step in making the determination that "undue experimentation" would have been needed to make and use the claimed invention is reached by weighing all the above noted factual considerations. *In re Wands,* 858 F.2d at 737, 8 USPQ2d at 737."

Here, the level of skill for one of ordinary skill in the art is high. The breadth of the claims is limited by the recital of at least 85% sequence identity. Furthermore, the invention is in a field of art which is highly advanced and in which considerable screening is undertaken as a matter of routine. In addition, the field is mature and has available to it combinatorial chemistry and high throughput screening methodologies to facilitate such

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[1] That some experimentation may be necessary to identify operative species does not constitute a lack of enablement. As the Federal Circuit has stated, "the key word is 'undue', not 'experimentation' " in determining whether pending claims are enabled. *Wands*, 8 U.S.P.Q.2d at 1405 (Fed. Cir. 1988). Indeed, a considerable amount of experimentation is permissible if it is merely routine, or if the specification in question provides a reasonable amount of guidance for practicing the invention.

screening on a large scale. Most importantly, as described above, the specification provides all the additional information needed to practice the invention in the claimed scope. In addition, the power and convenience of the research techniques available to one of ordinary skill in the art overwhelms the relatively limited uncertainty in the art by permitting a vast number of suitable fluorescent protein moieties to be made and screened with ease.

In particular, the Applicants note that the claims do not embrace all the possible variants of a green fluorescent protein having 85% sequence identity to that of SEQ ID NO:2, but reach only to such variants as at least comprise the particular advantageous substitutions set forth in the claims. Therefore, the Applicants submit that the claims are fully commensurate with the teachings of the specification and well comport with what may fairly regarded as their contribution to the art.

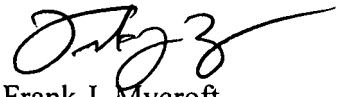
In light of the above Remarks and Amendments, Applicants believe that one of ordinary skill in the art can practice the invention as presently claimed according to the requirements of 35 U.S.C. §112, 1st paragraph. Accordingly, Applicants respectfully request that the above rejection be reconsidered and withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



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